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Rerouting 'coenzyme A' biosynthesis

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

2015

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Srinivasan, B. (2015). *Rerouting 'coenzyme A' biosynthesis*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.

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CHAPTER

Summarizing discussion and conclusion

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Adopted and modified from:

Article - Coenzyme A, more than 'just' a metabolic cofactor

Authors - Balaji Srinivasan and Ody C.M. Sibon

Published in Biochemical Society transactions. (2014) 42, 1075-1079.

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COENZYME A IS MORE THAN ‘JUST’ A METABOLIC COFACTOR

In all organisms, there are various metabolites that play a vital role to enable proper cellular metabolic processes. The human metabolome comprises a total of more than 25,000 identified endogenous metabolites¹. Such biological molecules or metabolites, mainly vitamins, amino acids, lipids, carbohydrates, nucleic acids, and nucleotides, are involved in important biological pathways and metabolic circuits¹⁻³. Alteration of metabolite homeostasis disrupts the physiology of cells, leading to various diseases². Recent studies advance our understanding that some metabolites are not only involved in cellular metabolism, but also have other molecular functions. It has become evident that similar to multifunctional ‘moonlighting proteins’, ‘moonlighting metabolites’ also exists. For example, nicotinamide adenine dinucleotide (NAD) was shown to regulate metabolism and also sirtuin deacetylase activity. This direct dual role of NAD could not be anticipated initially^{4,5}. Recent findings now also indicate that CoA, another metabolic cofactor, can be considered as being more than ‘just’ a metabolic cofactor, and altered CoA levels lead to severe and complex effects.

The importance of CoA and acyl-CoA in essential metabolic pathways like Krebs’s cycle and fatty acid β -oxidation was previously well-studied⁶. However, the role of acetyl-CoA in protein post-translational modification emerged more recently as an interesting focus in biological sciences⁷. Cellular citrate and pyruvate levels are primarily considered to regulate acetyl-CoA levels^{7,8}. Whereas the CoA biosynthesis pathway is crucial for the maintenance of free CoA levels, which subsequently can be converted into acetyl-CoA and thereby can serve, as an acetyl source. In addition to the influence of CoA levels on metabolism and protein-acetylation levels, recently the metabolic cofactor CoA has shown to influence other cellular functions as well. It was demonstrated that CoA can bind directly to calcium/calmodulin-dependent protein kinase II (CaMKII) and thereby activates CaMKII in *Xenopus laevis* oocytes promoting oocyte survival via phosphorylation and inactivation of caspase-2⁹. Moreover, evidence is presented that this is a conserved mechanism which is also present in mammalian oocytes⁹. These data reveal a signaling function of CoA independent from its role in cellular metabolism (Figure 1).

Based on the initial discoveries and the recent evidence of diverse CoA regulated cellular processes, it is evident that the maintenance of proper cellular CoA biosynthesis is vital. However, there is a lack of detailed research studies to understand the consequences of impaired *de novo* CoA biosynthesis. Here in this chapter, we will address the important findings presented in this thesis about consequences of impairment of the CoA biosynthesis pathway and also we will discuss how this is related to the pathophysiology of Pantothenate Kinase-Associated Neurodegeneration.

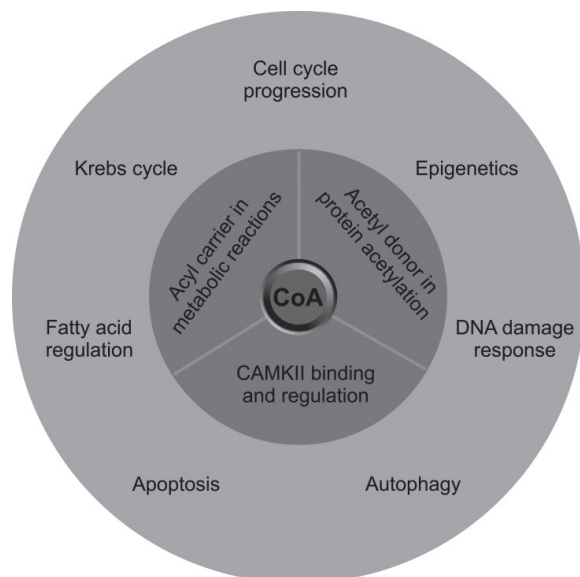


Figure 1: Schematic representation of diverse roles of CoA in cellular physiology.

Coenzyme A biosynthesis pathway and neurodegenerative diseases

The *de novo* CoA biosynthesis is carried out by the action of specific enzymes in several steps starting from pantothenate¹⁰. Pantothenate is present in sufficient quantities in the diet, and bacteria in human intestines also produce it. The enzymatic steps of the canonical CoA *de novo* biosynthesis route are well conserved and genes encoding for these enzymes have been identified in various organisms^{11–16}. CoA regained new attention after the discovery that one of the CoA biosynthetic enzymes ‘pantothenate kinase’ (or PANK) was found to cause a neurodegenerative disease characterized with very specific features. After the discovery of the causative gene ‘pantothenate kinase 2’ in 2001, the disease was named Pantothenate Kinase-Associated Neurodegeneration (PKAN)¹⁷.

PKAN is a subform of Neurodegeneration with Brain Iron Accumulation (NBIA), which is a group of disorders all characterized by iron accumulation in the brain¹⁸. Mutations in several seemingly unrelated genes (including pantothenate kinase 2) leads to a specific subform of NBIA^{19,20}. PKAN patients show the clinical manifestation of a progressive loss of extrapyramidal and pyramidal functions, and patients also often suffer from dystonia, dysarthria, and intellectual disabilities²¹. The identification of the causative gene for PKAN indicated a clear link between neurodegeneration and CoA *de novo* biosynthesis and suggests that the intact homeostasis of CoA levels is highly essential for normal brain function. However, to date, the pathophysiology of this disease is largely unknown, a therapy is lacking and how impaired *de novo* biosynthesis of CoA leads to a specific subform of NBIA is still unclear.

***Drosophila* to understand the link between Coenzyme A and neurodegeneration**

In order to investigate functions of CoA and consequences of impaired *de novo* CoA biosynthesis, several model organisms have been used, such as *Arabidopsis thaliana*²², mice^{23,24}, *Schizosaccharomyces pombe*²⁵ and *Drosophila melanogaster*^{11,14,26}. Here we will focus on the multicellular eukaryote model organism *Drosophila melanogaster* (fruit fly) and its potential aid in understanding the link between CoA and neurodegeneration will be summarized and discussed.

In 2001, a few months before PANK2 was identified as the causative gene for PKAN, Wasserman and coworkers described the phenotype of *Drosophila melanogaster* carrying mutations in the gene *fumble*, coding for pantothenate kinase¹¹. The *fumble* gene is also referred to as *dPANK/fbl* (*Drosophila pantothenate kinase/fumble*)¹¹. Although the researchers focused in this study on the male sterile phenotype, they also noticed the locomotor impairment of the mutant flies. Subsequently, they named the gene and the mutant after this locomotive phenotype; '*fumble*'. Wasserman and co-workers, therefore, were the first who revealed the link between impaired pantothenate kinase function and locomotor abnormalities. In addition to the locomotor defects, in *dPANK/fbl* mutant flies a decreased number of mitotic neuroblasts and an increase in abnormal (lagging chromosomes and anaphase bridges) mitotic chromosomes were observed¹¹. In addition, spermatocytes of *dPANK/fbl* mutant flies also showed defects in cytokinesis and chromosome segregation. Nevertheless, why mutations in *dPANK/fbl* lead to such specific phenotypes is not known yet. Later in the same year, the Hayflick laboratory identified human PANK2 as a causative gene for the severe autosomal recessive neurodegenerative disorder PKAN, further establishing a link between impaired CoA biosynthesis and abnormal locomotor functions¹⁷. However, PKAN is still surrounded by unresolved basic questions, such as: why do patients with mutations in PANK2 suffer from neurological symptoms, what are the mechanisms behind the specific symptoms and why is especially the nervous system affected?

Drosophila melanogaster is a versatile and genetically manipulatable model organism. It is widely used to study basic mechanisms in life sciences at various levels (molecular, cellular, tissue, developmental and behavioral) and it is also used as a model to study human diseases²⁷⁻²⁹. Similarly to many diseases with a genetic cause, a *Drosophila* PKAN model was explored, and *dPANK/fbl* mutants were further investigated to understand the link between neurodegeneration and CoA biosynthesis. Firstly, the *Drosophila* genes coding for enzymes required for the *de novo* biosynthesis of CoA were annotated by *in-silico* analysis and 5 *Drosophila* loci were identified coding for structural orthologs of PANK (*dPANK/fbl*), *PPCS* (*dPPCS*), *PPCDC* (*dPPCDC*) and a bifunctional *PPAT-DPCK* (*dPPAT-DCPK*; also called *dCOASY*) and a single *dDPCK*¹⁴. Moreover, it has been shown that *Drosophila* mutants carrying mutations in *dPANK/fbl*, *dPPCS* and *dPPAT-DCPK* show a comparable phenotype, strongly

suggesting that impaired CoA biosynthesis causes this shared phenotype¹⁴. All mutants show abnormal locomotor function (impaired climbing and flying behavior) which worsens upon ageing, a decreased life span, abnormal lipid homeostasis, increased sensitivity to Reactive Oxygen Species (ROS) inducing agents, increased apoptotic cells in the brain and increased sensitivity to DNA damaging agents¹⁴. Moreover, consistent with the previous results from Wasserman and co-workers¹¹ all investigated *Drosophila* CoA mutants show increased number of cells with abnormal mitotic chromosomes in larval brains. The phenotypes of all *Drosophila* CoA mutants were comparable. However, the severity of the phenotypes varied between the mutants, most likely because the mutants are hypomorphs, and, therefore, the rest-activity of each affected enzyme may be different.

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The use of *Drosophila dPANK/fbl* mutant made it possible for us to investigate whether the impaired function of *dPANK/fbl* actually leads to decreased levels of CoA. If so, this would suggest that decreased levels of the end product CoA is the primary cause of the reported defects. To investigate whether CoA biosynthesis is affected, we first measured CoA levels in both *dPANK/fbl* fly mutants and also in *dPANK/fbl* (by RNA-interference) depleted *Drosophila* cultured cells. The results presented in this thesis evidently shows that impaired *dPANK/fbl* activity leads to decreased levels of total CoA (Chapter 2)³⁰. More interestingly, in a selective compound screening, pantethine was found to replenish the decreased CoA levels and thereby partly rescued life span, neurodegeneration and locomotor defects in *dPANK/fbl* mutant flies (Chapter 2)³⁰. These data were the first to suggest that decreased levels of CoA may be causative of the observed neurodegenerative symptoms using a eukaryotic PKAN model system. These results also suggested that patients who carry mutations in other CoA biosynthesis enzymes may also suffer from a comparable disorder like PKAN. Indeed, the recent finding that the mutation in the gene coding for the last enzyme in the CoA biosynthesis pathway, PPAT-DPCK or Coenzyme A synthase (COASY), causes a new form of NBIA, referred to as CoPAN, further indicated that impairment of CoA homeostasis is strongly associated with neurodegeneration³¹. More detailed research needs to be performed to investigate whether the affected brain regions in PKAN and CoPAN show decreased CoA levels. Like vitamin-B5, Pantethine is also a known substrate for pantothenate kinase. Nevertheless, the restoration of decreased CoA levels in *dPANK/fbl* mutants with pantethine indicated a possible PANK-independent alternative route parallel to the canonical *de novo* CoA biosynthesis route (Chapter 2; Figure 2)³⁰. Moreover, vitamin-B5 did not rescue the *dPANK/fbl* mutant phenotype. These results indicate the presence of an unknown kinase able to phosphorylate pantetheine, or suggests the possibility that the residual PANK enzyme present in the hypomorph *dPANK/fbl* mutants is having a high affinity to pantethine. More detailed research is therefore needed to understand the working mechanism of pantethine.

PKAN and CoPAN in relation to mitochondrial integrity

The next question that arises is: how do decreased levels of CoA lead to neurodegeneration? It is yet unresolved whether decreased levels of CoA only cause metabolic defects or also lead to defects in non-metabolic processes in cells. Studies in mice showed that decreased levels of CoA influence the metabolic state of an organism^{23,32-35}. Therefore, the most likely underlying cause of PKAN and CoPAN is speculated to be abnormal metabolic reactions. The link between impaired cellular metabolism, mitochondrial dysfunction and severity of many neurodegenerative diseases is broadly recognized³⁶⁻³⁸. However, the debate of whether mitochondrial dysfunction is a primary cause or consequence of neurodegeneration remains. PKAN and CoPAN share similarities regarding mitochondria. The mitochondrial localization of both PANK2 and COASY suggests that mutations in PANK2 and COASY, lead to impaired mitochondrial function, and this may lead in turn to neurodegeneration^{31,39,40}. Our research data listed in this thesis also revealed that in both dPANK/fbl mutant flies and also in dPANK/fbl depleted cultured *Drosophila* cells, mitochondria are severely affected (chapter 2)³⁰. Moreover, it was also demonstrated that PANK2 downregulation in the human cell line (HEK293), resulted in impaired mitochondrial activity (chapter 2)³⁰. These results are in-line with the recent studies performed in mice. HoPan treatment induced both CoA depletion and severely swollen mitochondria in the mice liver³⁵. Moreover, in neurons derived from PANK2 KO mice, swollen mitochondria and defects in mitochondrial membrane potential were reported⁴¹. Concisely, all the above information strongly corroborated the link between PKAN and mitochondrial dysfunction. However, whether such a link also exist in CoPAN needs yet to be verified. Moreover, the impairment in CoA biosynthesis can also affect other functions independent of mitochondrial dysfunction.

Coenzyme A and its influence on protein-acetylation levels

The influence of altered CoA homeostasis on other than metabolic reactions became evident by a series of experiments, demonstrating that acetyl-CoA levels influenced protein acetylation. Acetyl-CoA provides the acetyl group, which is necessary for acetylation of proteins mediated by acetyltransferases (HAT/KAT's). Protein-acetylation is a post-translational modification influencing protein functions to a large extend^{42,43}. In *Saccharomyces cerevisiae* or budding yeast, it is reported that acetyl-CoA synthetase mutants have decreased levels of histone acetylation⁴⁴. Reduced levels of acetyl-CoA evoked by inactivation of the budding yeast ortholog of AMP-activated protein kinase (AMPK) also resulted in decreased histone acetylation, reduced fitness and stress resistance⁴⁵. In addition, two recent studies also showed a link between acetyl-CoA and autophagy. In budding yeast, it was demonstrated that acetyl-CoA is a suppressor of autophagy and down-regulation of acetyl-CoA synthetase in flies enhanced autophagy and increased life span⁴⁶. The second manuscript showed in mice and human cells that starvation resulted in decreased levels of acetyl-CoA, a reduction of acetylation of cytoplasmic proteins and induction of autophagy⁴⁷. When acetyl-CoA

levels were manipulated under these conditions and kept at a higher level, autophagy was suppressed. This suppression of autophagy was dependent on EP300 acyltransferase activity, suggesting acetyl-CoA levels regulate EP300 activity⁴⁷. Acetyl-CoA levels can also influence cell cycle progression because, in budding yeast, it was demonstrated that acetyl-CoA promotes acetylation of histones in the regulatory domain of the G1 cyclin CLN3 and cell cycle progression is promoted⁴⁸. Acetyl-CoA levels are not the only influencing factor for protein acetylation, as manipulating CoA levels itself can also regulate protein acetylation levels. We showed evidence that the decrease in CoA levels in both *Drosophila in vitro* and *in vivo* PANK model systems are associated with decreased levels of histone and tubulin acetylation. Moreover, restoration of CoA levels with pantethine reverted this phenotype (chapter 3; Figure 2)⁴⁹. Together these data indicated a strong link between CoA and/or acetyl-CoA in regulating acetylation of specific proteins.

Link between decreased histone and tubulin acetylation and neurodegeneration

Decreased histone and tubulin acetylation levels are associated with neurodegenerative diseases, and, therefore, the decreased levels of protein acetylation may explain part of the neurodegenerative phenotype of *dPANK/fbl* mutant flies. There is a strong link between cognitive function, motor neuron function, and histone acetylation^{50,51}. Fewer but nonetheless compelling studies reported the association between tubulin acetylation and neuronal function. Tubulin hyperacetylation protects against axonal degeneration⁵² and tubulin acetylation is required for the touch response in *C.elegans* (chapter 3)⁴⁹. In APPPS1-21 mice (a mouse model for Alzheimer's disease), levels of acetylated tubulin are decreased and restored in the presence of knockdown of the deacetylase Hdac6. In these APPPS1-21-Hdac6^{-/-} mice improvement of associative and spatial memory is observed compared to APPPS1-21 mice⁵³.

These data showed that the proper neuronal function requires normal levels of tubulin and histone acetylation. Therefore, the decreased levels of histone and tubulin acetylation, as observed in the *dPANK/fbl* mutants, could partly explain the neurodegenerative phenotype. The addition of valproic acid restored the decreased histone and tubulin acetylation levels and partly restored the locomotor function of mutant *dPANK/fbl* larvae, suggesting that impaired acetylation also underlies the observed phenotypes (chapter 3)⁴⁹. *dPANK/fbl* mutants also show increased sensitivity to DNA damaging and increased accumulation of DNA damage¹⁴. The impairment in histone acetylation could also explain such increased sensitivity, as acetylation of histones regulates DNA damage responses in various organisms^{43,54-58}. In *dPANK/fbl* depleted cultured *Drosophila* cells, after ionizing radiation, kinetics of histone acetylation are abnormal compared to control cells, which coincided with decreased survival of the cells after ionizing radiation. TSA rescued the decrease in protein acetylation and rescued partly the sensitivity (chapter 3)⁴⁹. Therefore, it may be possible that part of the neurodegenerative phenotype

observed in *Drosophila* CoA mutants is because of defects in DNA damage repair pathways, causing accumulation of damaged DNA in the brain. Impaired DNA damage repair is associated with neurodegeneration and several human diseases such as Ataxia Telangiectasia (ATM), AT-like Disorder (AT-LD), Nijmegen Breakage syndrome (NBS) and others^{59,60}. Although it is tempting to hypothesize that accumulation of damaged DNA in non-dividing neuronal cells leads to cell death and tissue loss, this order of events has not been convincingly demonstrated and how DNA damage leads to neurodegeneration is an unresolved puzzle⁶¹.

Alternative source for Coenzyme A biosynthesis and its therapeutic use for PKAN

In contrast to vitamin-B5, pantethine has a prompt rescue potency in restoring CoA levels and reverting acetylation defects in PKAN model systems (Chapter 2 and 3). Moreover, pantethine is reported to revert some of the disease phenotypes in other neurodegenerative disease models, like in Parkinson disease^{62,63}. However, the possible clinical use of pantethine as a rescuing molecule in patients may be limited because pantetheinases or vanins hydrolyse pantethine rapidly into cysteamine and pantothenate⁶⁴⁻⁶⁶. Pantetheinases are present ubiquitously in humans, especially in the intestine and are also excreted in their active form in the circulation. Although pantethine treatment has been shown to have beneficial effects in PANK2 KO mice reversing the disease phenotype induced by ketogenic diet, the rescue effect is thought to be mainly due to the anti-oxidant role of cysteamine⁶⁷. In contrast to pantethine, cysteamine did not revert the phenotype in the *Drosophila* PKAN model system (unpublished data). To improve pantethine stability and thereby improve its applicability in humans as a rescue agent for PKAN, we synthesized a novel pantetheine derivative with a lipophilic thiobutyl triphenylphosphonium (TBTP) cation (chapter 4; Figure 2). Moreover, the use of such a lipophilic group will increase the chance of effective cell membrane and blood brain barrier permeability and in addition act as a selective targeting utility to reach mitochondria. TBTP-pantethine indeed showed increased membrane affinity⁶⁸⁻⁷⁰. However, such modification of pantethine did not increase the passive membrane permeability as compared to pantethine. Moreover, TBTP coupling did not increase the stability of pantethine, as pantetheinases present in fetal calf serum still degraded the TBTP-pantethine (Chapter 4; Figure 2). Although the identification of PANK-independent alternative CoA biosynthesis from pantethine changes the concept of vitamin-B5 being the only substrate to form cellular CoA, the instability of pantethine narrows its pharmacological benefits. It also clearly indicated the need to explore other possible sources for cellular CoA, which might be of therapeutic use to treat PKAN effectively.

CoA has been considered physiologically inert regarding functions outside the cell, and this molecule was never envisioned to be able to pass through the cell membrane, due to its bulky and highly electronegative nature. However, in an attempt to revert the protein acetylation defect induced by PKAN impairment in a *Drosophila in vitro* model,

direct supplementation of CoA in the external cell media revealed a complete rescue effect (Chapter 3; Figure 2)⁴⁹. Moreover, CoA supplementation along with statins has been shown to have therapeutic benefit in treating hyperlipidemia^{71,72}. These striking results made us

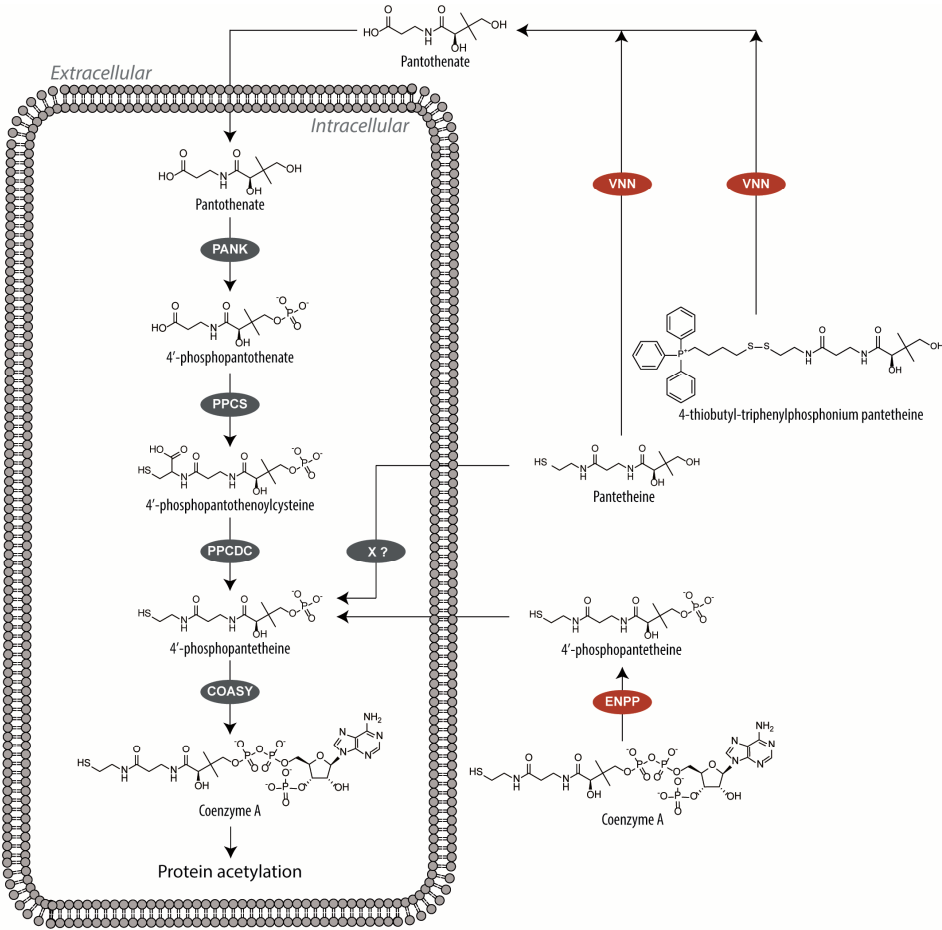


Figure 2: Over-view of research findings summarized in this thesis.

De novo CoA biosynthesis utilizing extracellular pantothenate and subsequently processed by pantothenate kinase (PANK), 4'-phosphopantetheincysteine synthetase (PPCS), (R)-4'-phospho-N-pantetheincysteine decarboxylase (PPCDC), and the bifunctional 4'-phosphopantetheine adenyltransferase/dephospho-CoA kinase (COASY) to form CoA. Pantetheine by-passes PANK and is processed by an unknown kinase/protein (X?) contributing to CoA biosynthesis (Chapter 2). Cellular CoA levels regulate the protein acetylation homeostasis inside the cell (Chapter 3). Pantetheinases or vanin (VNN) degrade both pantetheine and 4-thiobutyl-triphenylphosphonium pantetheine to pantothenate in the serum (Chapter 4). External supplemented CoA is converted into 4'-phosphopantetheine by ectonucleotide-pyrophosphatases (ENPP) in the serum, 4'-phosphopantetheine crosses the plasma membrane via passive diffusion and contributes to CoA biosynthesis in a COASY dependent manner (Chapter 5).

investigate whether such direct effects of CoA exist and if so what is the working mechanism behind it. To aid our study in a more precise way, we developed a sensitive chromatography method with selectivity for CoA and other related thiol molecules (Chapter 3 and 5).

The evidence presented in Chapter 5 of this thesis demonstrated that cells and organisms are indeed able to acquire CoA not only via the uptake of vitamin B5 but also via the direct supplementation of exogenous CoA. Our experimental data showed that CoA needs to be hydrolyzed extracellularly by exo-enzymes into 4'-phosphopantetheine (PPanSH), which is a biologically stable molecule and is taken up by cells. Intracellularly, PPanSH is converted back to CoA by the bifunctional enzyme COASY. This newly identified non-canonical source for CoA is independent of PANK and PPCDC. As described in Chapter 5, external supplementation of CoA, but not vitamin B5, rescued various phenotypes induced by impaired PANK and PPCDC in *Drosophila* model systems. The rescue effect of CoA in a validated *dPANK/fbl null* mutant and in a severely affected *dPPCDC Drosophila* mutant, but not *dCOASY* mutants, clearly revealed a potent working mechanism of CoA supplied via fly food. We also provided evidence that this mechanism is prevalent among diverse eukaryotic organisms and cells, including *C.elegans*, human cells and in mice. The proposed mechanism thus suggests a net flow of CoA via PPanSH between cells and between membrane-bound cellular compartments. Moreover, the rescue potency of CoA in reverting the female sterility in *dPPCDC* mutants clearly supports that the beneficial effect of PPanSH obtained from CoA is far reaching within an organism and is crossing various cellular barriers. In contrast to the instability of pantethine in human serum, PPanSH obtained via external CoA is found to be stable which indicates its promises to be an effective non-canonical substrate for CoA biosynthesis obtained via the circulation. The confirmation of endogenous PPanSH in mouse serum by both HPLC and mass spectrometry, substantiates the natural occurrence of this alternative source route in higher eukaryotes for CoA biosynthesis (Chapter 5). Exogenous CoA or PPanSH might not be beneficial for CoPAN therapy, as this alternative route requires active COASY. Nevertheless, CoA or PPanSH might provide a promising treatment possibility for PKAN.

Concluding remarks and perspectives

With the knowledge that CoA is not only a key molecule in metabolism playing a role in over 100 metabolic reactions, but that CoA also modulates the epigenome and at least activates one specific kinase (CaMKII)⁹, the influence of this molecule in health and disease is most likely larger than ever anticipated. Further detailed investigation is yet required to understand whether impaired metabolism, abnormal protein acetylation or impaired activation of CaMKII or possible other proteins or a combinatorial effect causes the specific disease characteristics of PKAN and CoPAN. It is also important to understand the effects of mutations of *PANK2* and *COASY* with regards to their activity in both CoA biosynthesis but also in mitochondrial structural integrity. Since not all the mutations leading to PKAN correlates with loss of *PANK2* activity^{73,74} and with the use of CoPAN patient fibroblasts evidence was

presented that CoA is still being produced³¹, mitochondrial dysfunction could be one of the leading cause of neurodegeneration. Hence, it is necessary for the future to perform further in-depth experiments to reveal precisely the link between the multifaceted influence of CoA and its related diseases, including PKAN and CoPAN.

It is also important to elucidate additional alternative possible ways to manipulate cellular CoA levels. *In vitro* studies have indicated that small molecules like palmitoyl carnitine and oleyl carnitine can activate PANK^{75,76}. However, this has never been confirmed *in vivo*. Exploiting such small molecules as activators or repressors will be useful in our research to find possibilities to be able to manipulate CoA levels and this knowledge will be helpful to understand the role of CoA in cellular physiology and may lead to possible ways to treat PKAN and CoPAN. High throughput screening to identify regulators of the promoters of the genes coding for CoA biosynthesis enzymes could also be a useful approach⁷⁷. Moreover, the use of pantethine or TBTP-pantetheine as novel alternative substrates for CoA production can be substantiated by combining them with pantetheinase inhibitors^{78,79} to achieve clinical benefit.

Moreover, the use of HDAC inhibitors like TSA alone or in addition to pantethine or pantetheine derivatives might also provide efficient benefits compared to treatment with pantethine or pantetheine derivatives only. The new breakthrough in deciphering the working mechanism of externally supplemented CoA via PPanSH has broadened the scope of CoA research in both basic cell biology and in disease pathophysiology. Our experimental proof of PPanSH as a crucial molecule in reverting the PKAN phenotype may be a physiologically relevant process because we could detect endogenous PPanSH in fresh mouse serum. In clinical studies, CoA alone or with the addition of statins showed a beneficial effect in lowering plasma triglyceride levels in patients with moderate dyslipidemia and thereby acted as a natural hypolipidemic drug with no obvious adverse effect detected^{71,72}. Our findings suggest that the above mentioned CoA mode of action in hyperlipidemia is via PPanSH. Recently, the *de novo* CoA biosynthesis pathway in microbes also obtained interest because of the fact that small molecules can be developed acting as novel antimicrobial drugs by specifically targeting the microbial CoA biosynthesis enzymes^{80,81}. This is possible because prokaryotic CoA biosynthesis pathway enzymes vary in their sequences and structural integrity compared to the eukaryotic enzymes⁸⁰⁻⁸². In addition, the development of CoA/PPanSH antimetabolites that can selectively inhibit the activity of parasite and microbial CoA biosynthesis enzymes and thereby reduce CoA levels and/or interfere with the species-specific CoA dependent processes will be promising to treat parasite and microbial infections^{80,81}. Overall, this thesis summarized the diverse influence/function of CoA, apart from its most classically known metabolic role. It also reports about in-depth experimental outcomes to understand the role of impaired *de novo* CoA biosynthesis in cellular physiology. In addition, the finding of a possible alternative source for CoA biosynthesis like pantethine, PPanSH, and CoA provides a promising start to design a therapy for PKAN and other CoA related diseases.

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